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# EXTRAVASCULAR ALBUMIN IN BONE TISSUE

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SUMMARY

1. The amount of albumin in extravascular tissue fluid in bone, kidney, intestine, skin and muscle and in plasma of young rabbits has been measured by radial immunodiffusion.

2. The majority of extravascular albumin in kidney, intestine, skin and muscle is exchangeable with plasma albumin, whereas in bone, only the proportion which is in tissue fluid is readily exchangeable; the remaining fraction in calcified matrix is more permanently fixed.

3. About 27% of the albumin in young bone is in tissue fluid, about 57% in calcified matrix and about 16% is intravascular. The total amount of extravascular albumin per unit mass of bone is similar to that found in soft tissues.

4. The volume of intravascular plasma in tissues was determined in two ways: from $^{51}$Cr-erythrocyte radioactivity and the venous haematocrit and from the '5 min $^{125}$I-fibrinogen space'.

5. The rate of egress of albumin from blood vessels has been estimated from the initial slope of the ratio of extravascular radioactivity in the tissue to plasma radioactivity plotted against time after injection of $^{125}$I-albumin.

6. The rate of clearance of the albumin in extravascular tissue fluid in bone is approximately once every hour. This is more rapid than in skin and muscle, comparable with intestine and less rapid than in kidney.

7. The amount of albumin incorporated into calcified matrix of bone per day is calculated to be less than 0·5% of the total albumin passing through the tissue fluid of bone per day.

## INTRODUCTION

Albumin is the most abundant plasma protein and quantitatively is an important component of extravascular tissue fluid. According to current models of albumin kinetics the protein escapes from the capillaries in

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different tissues and circulates through extravascular tissue before being returned to blood, mainly via the lymph (Katz, Sellers, Bonorris & Golden, 1970). Albumin has many physiological roles yet relatively few investigations of its turnover in different tissues have been made.

In a recent study we have found that albumin is a constituent of the organic matrix of bone (Triffitt & Owen, 1973; Ashton, Triffitt & Herring, 1974). Using $^{125}$I-labelled albumin it has been shown that albumin, immunologically identical to plasma albumin, is incorporated into calcified matrix from the tissue fluid at sites of bone formation (Owen, Triffitt & Melick, 1973). In the present paper further studies show that, whereas the majority of extravascular albumin in soft tissues is exchangeable with plasma, in bone only the proportion in extravascular tissue fluid is readily exchangeable, the fraction in calcified matrix being more permanently fixed. Investigations of the amount of albumin in extravascular tissue fluid in bone, kidney, intestine, skin and muscle and its passage from intravascular to extravascular space have been made in rabbits using $^{125}$I-labelled albumin and a dual isotope technique similar to that used by Studer & Potchen (1971). A major objective was to investigate the rate of turnover of extravascular albumin in bone and the relative importance of its incorporation into calcified matrix. It was found that the amount of albumin incorporated into calcified matrix in the process of bone formation is small compared with the total albumin passing through the tissue fluid of bone.

Interstitial fluid in bone is in close proximity to the surfaces of bone mineral and consequently may be important for plasma ion homoeostasis. A previous study of the ionic composition of bone tissue fluid using an indirect method suggests that this differs from an ultrafiltrate of plasma (Neuman, 1969). Because of the difficulty in obtaining samples of tissue fluid in bone it has not been possible, so far, to make direct studies of its composition and turnover and the kinetic methods used in the present work give information that would be difficult to obtain otherwise.

## METHODS

Dutch rabbits, 3½–4 weeks old, average weight 400 g (young), and 1–2 years old, average weight 2·5 kg (adult), were used. All rabbits were fed a standard laboratory diet. Electrophoretically pure rabbit albumin was obtained from Koch-Light laboratories, Colnbrook, Bucks. [$^{125}$I]Iodide (80–140 mc/ml.), sodium chromate ($^{51}$Cr) solution (100–300 $\mu$c/mg of Cr) and $^{125}$I human fibrinogen (100 $\mu$c/mg) were obtained from the Radiochemical Centre, Amersham.

Iodination of albumin with $^{125}$I and labelling of erythrocytes with $^{51}$Cr was performed by methods described previously (Owen *et al.* 1973; Triffitt & Owen, 1973). The [$^{125}$I]albumin solution, concentration 0·35 mg/ml., with a specific radioactivity of about 0·5 mc/mg and labelled with about 0·03 I atoms per molecule,

contained less than 1·0 % non-protein bound iodide and this was reduced to negligible levels by dialysis before injection. Albumin iodinated with less than 0·5 I atoms per molecule is eliminated from the body at an identical rate to albumin labelled biosynthetically using radioactive amino acids (Anker, 1960; McFarlane, 1964). A preliminary experiment confirmed that the turnover in plasma of the $^{125}$I-albumin was identical with that of biosynthetically $^{3}$H-labelled albumin in the adult rabbits.

All injections were intravenous via the ear vein. Young and adult rabbits were given $^{125}$I-albumin in isotonic saline (0·06 mc and 0·02 mc/kg body wt. respectively), and killed at intervals from 5 min to 7 days after injection. A weighed portion of $^{51}$Cr-erythrocytes in isotonic saline (0·5 ml./kg body wt.) with a specific radioactivity of about 2 $\mu$c/ml. was injected into each animal 30 min before death.

*Radioactivity measurements.* All samples were assayed for radioactivity using a two-channel, well-type, automatic $\gamma$-scintillation counter. Separation of $^{51}$Cr and $^{125}$I was accomplished by pulse height analysis and all counts were corrected for decay. In two animals from each age group blood samples were taken from the ear vein at intervals up to 7 days and blood radioactivity curves plotted. Samples of blood and plasma were assayed for each isotope. Pieces of cortical bone from tibia and femur cleaned of marrow and soft tissue (0·7 g), small intestine minus contents (0·2 g), skin minus hair (0·3 g), the adductor longus muscle from the femur (0·7 g) and one complete kidney (four pieces about 0·5 g each) were placed in vials, weighed, and counted for $^{125}$I and $^{51}$Cr. The radioactivity per gram of fresh tissue (cpm/g) for $^{51}$Cr and $^{125}$I was determined for each tissue.

*Tissue blood and plasma volumes.* Blood samples were obtained from the jugular vein and the venous haematocrit determined by using microhaematocrit tubes. The total body blood volume was determined from the $^{51}$Cr counts in blood by isotope dilution technique. The blood volume per gram of tissue was calculated from the $^{51}$Cr radioactivity in the tissue. Total body intravascular plasma volume and intravascular plasma volume per gram of tissue were calculated using the venous haematocrit.

*Tissue extraction.* On removal from the animal the tissues were placed immediately in 3 ml. 0·9 % NaCl solution in 0·004 M barbitone buffer, pH 7·0, at 4° C in the vials in which they were weighed and counted. The NaCl solution was changed 3 times at 24 hourly intervals and the extracts assayed for radioactivity.

*Precipitability of extracted radioactivity.* Trichloracetic acid (TCA) was added to 2 ml. portions of the NaCl extract and to plasma samples to a final concentration of 10 % (w/v). The precipitate was left at 4° C overnight and after centrifugation the supernatant liquid was decanted and assayed for radioactivity immediately while still cold. Prompt analysis is essential to avoid break-down and the results obtained for the proportion of $^{125}$I radioactivity which is non-precipitable are likely to be maximum values.

*Quantitation of albumin.* The amount of albumin in the NaCl extracts of tissues and in plasma was determined by single radial immunodiffusion (Mancini *et al.* 1965) as previously described (Owen *et al.* 1973). An anti-rabbit albumin was raised in guinea-pigs and a 2 % solution of the antiserum in 1 % agar gel used.

*Extravascular $^{125}$I-radioactivity.* The extravascular $^{125}$I-radioactivity per gram of tissue (EV $^{125}$I cpm/g), was determined for each tissue at different times after injection. This was obtained from the total $^{125}$I cpm/g tissue minus the intravascular $^{125}$I cpm/g tissue. The latter was calculated by using the intravascular plasma volume/g tissue and the plasma $^{125}$I radioactivity measurements.

*Quantitation of extravascular albumin.* Extraction of tissues with isotonic NaCl removes the majority of albumin from soft tissues (Katz *et al.* 1970 and present results). From young bone NaCl removes albumin from vessels and tissue fluid but not from calcified matrix (Owen *et al.* 1973). Hence the amount of extravascular

albumin in soft tissues and in bone tissue fluid is equal to the albumin content of the NaCl extract after the contribution due to plasma albumin in vessels has been subtracted.

*Tissue: plasma ratio.* This is defined as $\frac{\text{EV } ^{125}\text{I cpm/g tissue}}{^{125}\text{I cpm/g plasma}}$. It is expressed in 'grams of plasma equivalents' and is equal to the number of grams of plasma which contains the amount of $^{125}$I radioactivity to be found at that time outside vessels per gram of tissue. Following injection, the tissue: plasma ratio will increase with time and should reach an approximately constant value as a steady-state condition for exchange of labelled albumin between plasma and extravascular tissue is attained. For a particular tissue the time taken to reach a constant value will depend mainly on the rate of egress of albumin from the blood vessels and the size of the extravascular space. The ratio, as defined above in 'grams of plasma equivalents', can be expressed as mg albumin/g tissue using the values obtained for plasma albumin concentrations.

*Egress of albumin.* The amount of intravascular albumin which passes into extravascular tissue/g tissue.unit time has been obtained from the initial slope of the curve for the tissue: plasma ratio plotted against time after injection of $^{125}$I-albumin.

*Intravascular plasma in tissues.* The amount of intravascular plasma in tissues, as calculated from $^{51}$Cr-erythrocyte radioactivity, is based on the assumption that the tissue haematocrit is equal to the venous haematocrit. This may not be true (Swan & Nelson, 1971; Studer & Potchen, 1971) and therefore the space occupied in the tissue by $^{125}$I-fibrinogen 5 min after injection was determined for comparison with the plasma space calculated from $^{51}$Cr-radioactivity and with the space occupied by $^{125}$I-albumin 5 min after injection. Fibrinogen has a molecular weight of about 340,000 and a small extravascular space in the body (~20%) (Regoeczi, 1974). Hence there should be negligible escape of fibrinogen from the blood capillaries in most tissues within 5 min. Seven young rabbit litter-mates were injected with $^{51}$Cr-erythrocytes 30 min before death, four of them were injected with $^{125}$I-albumin 5 min before death and the other three with $^{125}$I-fibrinogen (0·06 mc/kg body wt.) 5 min before death. The 5 min $^{125}$I-albumin and $^{125}$I-fibrinogen spaces in $\mu$l. of plasma/g tissue were determined from $^{125}$I cmp/g tissue divided by $^{125}$I cpm/$\mu$l. plasma.

## RESULTS

*Blood and plasma volumes.* The total body intravascular blood and plasma volumes (ml./kg body wt. ± S.D., fifteen animals in each group), for young and adult rabbits are 51·1 ± 5·7, 35·2 ± 5·2 and 32·5 ± 7·8, 18·9 ± 4·8 respectively. For the different tissues the intravascular plasma volumes ($\mu$l./g tissue) are shown in columns *a* and *d* of Table 1. Bone of young rabbits is four times as vascular as that of adult rabbits; in other tissues there is no significant difference between the two age groups (Table 1). The volume of the 5 min $^{125}$I-albumin space is 1·7–4·4 times and of the 5 min $^{125}$I-fibrinogen space 1·3–3·2 times the plasma volume in the tissue as calculated from the $^{51}$Cr radioactivity (*b* and *c* of Table 1).

*Tissue extraction and TCA precipitability.* Following injection of $^{125}$I-albumin the percent of $^{125}$I-radioactivity removed in NaCl extracts of bone and soft tissues and the amount precipitable in extracts and in

plasma from young rabbits is shown in Table 2*a*. Similar results for bone and plasma of adult rabbits is shown in Table 2*b*. The amount of $^{125}$I-radioactivity which can be extracted from young bone decreases with time after injection of $^{125}$I-albumin and is only 25% by 3 days. This is because a large fraction of the $^{125}$I-albumin in bone at 3 days is in a band in calcified matrix where it was incorporated during bone formation and it is not

TABLE 1. Intravascular plasma measurements for different tissues

| | Young rabbits | | | Adult rabbits |
|---|---|---|---|---|
| Tissue | *a* | *b* | *c* | *d* |
| Kidney | 33·1 ± 8·1 (15) | 4·39 ± 0·51 (4) | 3·26 ± 0·36 (3) | 41·4 ± 10·1 (15) |
| Intestine | 8·9 ± 3·2 (15) | 2·80 ± 0·30 (4) | 2·08 ± 0·22 (3) | 5·1 ± 3·2 (15) |
| Skin | 8·3 ± 2·9 (15) | 1·85 ± 0·18 (4) | 1·75 ± 0·18 (3) | 6·8 ± 2·5 (6) |
| Muscle | 5·0 ± 1·5 (15) | 1·75 ± 0·17 (4) | 1·52 ± 0·14 (3) | 3·0 ± 0·9 (6) |
| Bone | 10·8 ± 3·1 (15) | 1·90 ± 0·20 (4) | 1·31 ± 0·14 (3) | 2·7 ± 0·6 (15) |

Mean ± S.D., no. of rabbits in brackets.

*a* and *d* intravascular plasma volume, μl./g tissue, calculated from $^{51}$Cr-erythrocyte radioactivity and venous haematocrit.

*b* ratio '5 min $^{125}$I-albumin space'/g tissue to intravascular plasma volume/g tissue, calculated from $^{51}$Cr-radioactivity and venous haematocrit.

*c* ratio '5 min $^{125}$I-fibrinogen space'/g tissue to intravascular plasma volume/g tissue, calculated from $^{51}$Cr-radioactivity and venous haematocrit.

removable by NaCl (Owen *et al.* 1973). In soft tissues in young animals about 90% of the radioactivity is removed by washing with NaCl and in adult bone about 80% at all times (Table 2*a* and *b*). Since there is comparatively little bone formation in adults most of the extravascular radioactivity in bone is in the exchangeable tissue fluid compartment. In both young and adult animals the TCA precipitability of the $^{125}$I-radioactivity in plasma is always close to 100%, whereas in the extracts of the tissues it is consistently lower (Tables 2*a* and *b*).

*Radioactivity measurements.* The fall-off in $^{125}$I-radioactivity in blood of young and adult rabbits following a single I.V. injection of $^{125}$I-albumin is shown in Fig. 1. Five samples were counted at each time and the S.D. was less than ±3%. For the tissues the EV $^{125}$I cpm/g in young and adult animals at different times after injection is plotted in Figs. 2 and 3 respectively. In all tissues, except young bone, EV cpm/g reach a maximum and then fall off approximately parallel with the fall-off in radioactivity in

blood (cf. Figs. 2 and 3 with Fig. 1). Curves of this shape suggest that the majority of the extravascular albumin in the tissue is exchangeable with plasma. Maximum labelling of the extravascular compartment is reached very rapidly in kidney (within 5 min) and at later times in intestine, adult bone, skin and muscle.

TABLE 2. Percent $^{125}$I-radioactivity in NaCl extract and percent which is TCA-precipitable in NaCl extract and in plasma (mean ± S.D. for three or more rabbits, average for two, no. of rabbits in brackets)

| Tissue | Time after injection | % in NaCl extract | % precipitable in NaCl extract | % precipitable in plasma |
|---|---|---|---|---|
| | | *a*. Young rabbits | | |
| Kidney | 5 min | | 92·3 ± 1·5 (3) | 98·8 ± 1·0 (3) |
| | 30 min | 92·0 (2) | 91·8 (2) | 98·4 ± 0·2 (3) |
| | 6 hr | | 89·8 (2) | 97·3 ± 0·9 (3) |
| | 24 hr | | 84·0 (2) | 97·8 ± 0·9 (3) |
| | 3 days | 90·0 ± 1·2 (5) | 78·7 ± 2·0 (3) | 95·7 ± 3·1 (5) |
| Intestine | 5 min | 92·9 ± 1·2 (3) | 94·7 ± 1·4 (3) | |
| | 30 min | 94·1 (2) | 95·3 (2) | |
| | 6 hr | | 93·1 (2) | |
| | 24 hr | | 82·8 (2) | |
| | 3 days | 93·0 ± 1·4 (5) | 78·2 ± 2·1 (3) | |
| Skin | 30 min | 93·9 ± 2·0 (3) | 86·8 ± 1·1 (3) | |
| | 3 days | 95·7 ± 1·9 (5) | 90·6 ± 2·8 (5) | |
| Muscle | 5 min | | 89·6 ± 2·1 (3) | |
| | 30 min | 93·2 (2) | 93·7 (2) | |
| | 3 days | 87·1 ± 2·2 (5) | 92·5 (2) | |
| Bone | 5 min | 91·3 ± 2·1 (3) | 94·8 ± 1·5 (3) | |
| | 30 min | 93·3 ± 4·4 (3) | 89·0 ± 3·5 (3) | |
| | 6 hr | 78·2 ± 4·0 (3) | 74·0 ± 5·5 (3) | |
| | 24 hr | 46·8 ± 4·5 (3) | 81·7 ± 3·8 (3) | |
| | 3 days | 25·2 ± 3·2 (5) | 77·5 ± 6·5 (5) | |
| | 7 days | 16·6 (2) | | |
| | | *b*. Adult rabbits | | |
| Bone | 20 min | 85·0 (2) | 91·6 (2) | 98·9 (2) |
| | 1 hr | 80·3 ± 3·3 (3) | 77·9 ± 6·9 (3) | 95·1 ± 6·9 (3) |
| | 6 hr | 83·9 (1) | 72·5 (1) | 98·0 (1) |
| | 24 hr | 81·8 (2) | 74·2 (2) | 98·2 (2) |
| | 3 days | 78·1 ± 3·8 (3) | 72·0 ± 4·4 (3) | 94·0 ± 4·8 (3) |

In contrast, in young bone EV radioactivity reaches a maximum and does not fall off appreciably during the period of the experiment (Fig. 2). This occurs because some of the $^{125}$I-albumin which enters extravascular tissue fluid is incorporated into calcified matrix and is not exchangeable with plasma. The fraction of extravascular radioactivity in bone tissue fluid can be estimated from the amount in the NaCl extract (Table 2*a*)

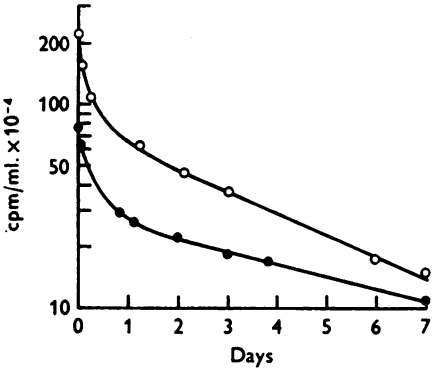


Fig. 1

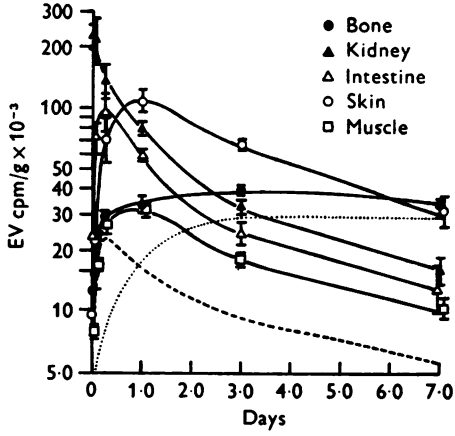


Fig. 2

Fig. 1. Radioactivity in blood following a single injection of $^{125}$I-albumin, in young ○ and adult ● rabbits. In all figures the continuous lines are drawn by eye.

Fig. 2. EV $^{125}$I-radioactivity/g tissue plotted against time after injection for different tissues in young rabbits. The dotted curves are EV cpm in calcified matrix/g bone (. . . . .), and EV cpm in tissue fluid/g bone (– – –), calculated from the data for the amount extracted by NaCl, see text.

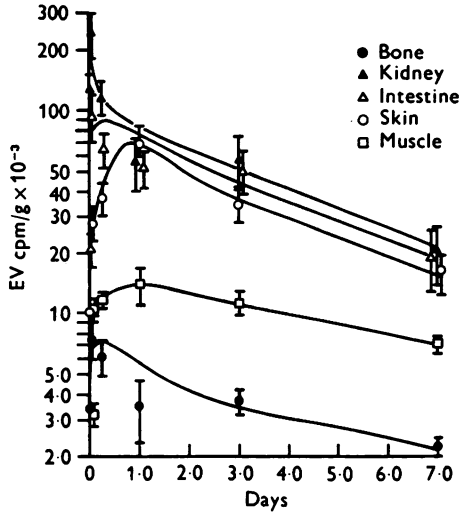


Fig. 3. EV $^{125}$I radioactivity/g tissue plotted against time after injection for different tissues in adult rabbits.

TABLE 3. Extravascular albumin (mg/g tissue), intravascular albumin (mg/g tissue), and egress of albumin from intravascular to extravascular space (mg/g tissue.hour) in young rabbits

| Tissue | 1 EV albumin (mg/g) | | 2 IV albumin (mg/g) | | 3 Egress of albumin (mg/g.hr) | |
|---|---|---|---|---|---|---|
| | *a* | *b* | *a* | *b* | *a* | *b* |
| Kidney | 6·7 ± 0·8 | 4·11 | 1·2 | 3·79 | 31·2 | 10·3 |
| Intestine | 4·3 ± 0·9 | 3·96 | 0·32 | 0·66 | 3·0 | 1·7 |
| Skin | 6·8 ± 0·6 | 6·58 | 0·30 | 0·52 | 1·1 | 0·41 |
| Muscle | 1·7 ± 0·2 | 1·61 | 0·18 | 0·27 | 0·56 | 0·28 |
| Bone | *0·83 ± 0·16 | *0·71 | 0·39 | 0·51 | 1·1 | 0·66 |

* Value for bone tissue fluid/g bone.

1, from radial immunodiffusion measurements on NaCl extracts of tissues from four rabbits, mean ± S.D.

2, calculated using results for intravascular plasma volumes (Table 1) and plasma albumin concentrations.

3, calculated from initial slope of graph for tissue:plasma ratio against time.

*a*, results calculated assuming plasma volume in tissue equal to that derived from $^{51}Cr$ radioactivity and venous haematocrit.

*b*, results calculated assuming plasma volume in tissue equal to the 5 min $^{125}I$-fibrinogen space.

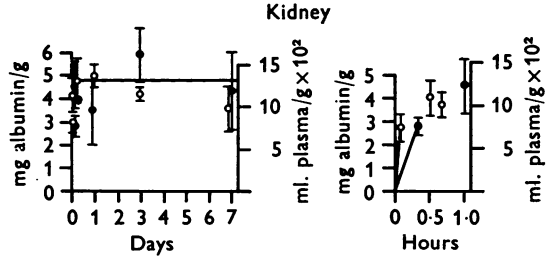


Fig. 4. Tissue: plasma ratios in young ○ and adult ● rabbits plotted against time after injection, i.e. $\frac{\text{EV } ^{125}I \text{ cpm/g tissue}}{^{125}I \text{ cpm/g plasma}}$ expressed as mg albumin/g tissue, left hand scale and ml. plasma/100 g tissue, right hand scale. Dashed curve in Fig. 8 on left is for bone tissue fluid in young rabbits, i.e.

$$\frac{\text{EV } ^{125}I \text{ cpm in tissue fluid/g bone}}{^{125}I \text{ cpm/g plasma}}.$$

The data are calculated using the intravascular plasma volume for the tissue obtained from the $^{51}Cr$ erythrocte activity and the venous haematocrit. The rate of egress of albumin from the blood vessels can be calculated from the initial slope which is shown in the right hand graphs.

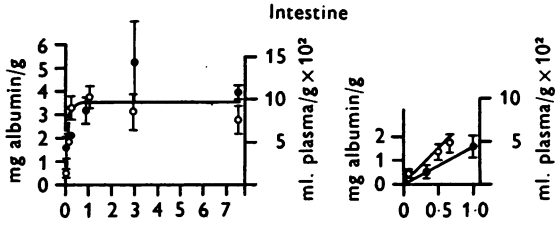


Fig. 5. As Fig. 4.

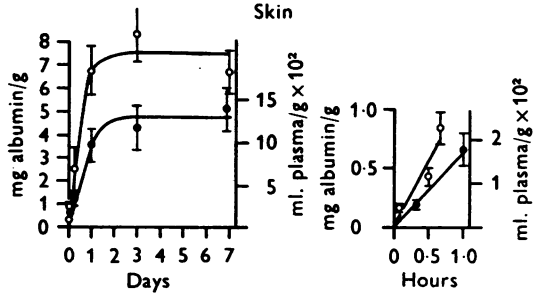


Fig. 6. As Fig. 4.

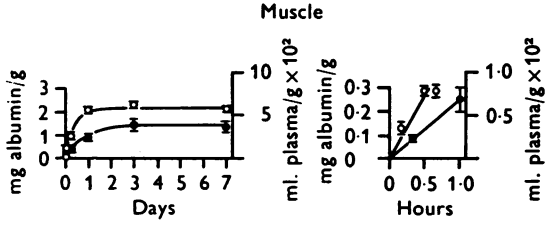


Fig. 7. As Fig. 4

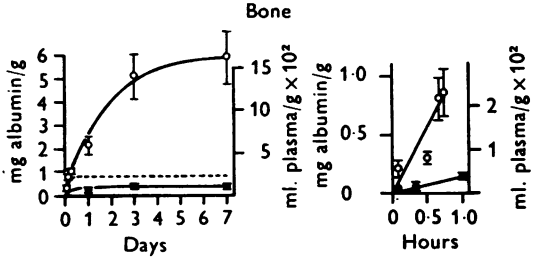


Fig. 8. As Fig. 4.

after allowing for the contribution due to intravascular radioactivity. Hence the EV cpm/g bone has been calculated for each of the two compartments; 'bone tissue fluid' and 'calcified matrix' (interrupted curves Fig. 2). The EV radioactivity in bone tissue fluid (Fig. 2) reaches a

maximum within a few hours after injection and then falls off approximately parallel with plasma radioactivity suggesting that the radioactivity in bone tissue fluid is exchangeable with plasma similar to the situation in soft tissues. The rate of uptake of labelled material into calcified matrix decreases as the levels of radioactivity in plasma and tissue fluid fall.

*Intravascular albumin.* The concentration of albumin in plasma in young and adult rabbits, fifteen animals in each group, measured by radial immunodiffusion was found to be $36{\cdot}5 \pm 2{\cdot}2$ and $41{\cdot}0 \pm 2{\cdot}7$ mg/ml. respectively.

*Extravascular albumin.* The amount of extravascular albumin determined from radial immunodiffusion measurements of the NaCl extracts of the tissues, is shown in column 1 Table 3, in mg/g tissue. The values in column 1*a* were calculated using the plasma volumes estimated from $^{51}$Cr-radioactivity in the tissue and those in column 1*b* were derived from these by assuming that the plasma space in the tissue is equivalent to the 5 min fibrinogen space using the results in Table 1*c*. Intravascular albumin/g tissue calculated in a similar way is given for comparison in columns 2*a* and *b* of Table 3.

*Tissue: plasma ratio.* This is plotted with time after injection expressed in mg albumin/g tissue and also in 'ml. plasma equivalents/100 g tissue' for comparison with other published work where these units are used (left and right hand scales respectively Figs. 4 to 8). In both kidney and intestine the tissue: plasma ratio reaches a constant value very rapidly (Figs. 4 and 5). In the case of skin, muscle and bone, equilibrium conditions between plasma and extravascular tissues are established more slowly, about one to three days after injection (Figs. 6, 7 and 8). In young bone some $^{125}$I-albumin becomes fixed in calcified matrix and this explains the continued increase of the tissue: plasma ratio during the period of the experiment. The tissue: plasma ratio has been calculated for tissue fluid in young bone using the results for extraction with NaCl (Table 2*a*) and it reaches a constant value by a few hours after injection, interrupted curve in Fig. 8. An estimate of the amount of extravascular albumin/g tissue can be obtained from the constant values for the tissue: plasma ratio, provided a correction is made for non-protein bound radioactivity and the time lag in return of albumin from extravascular to intravascular space in the different tissues. This would lower the constant values in Figs. 4–8 by about 10% for skin and muscle, 20% for kidney and intestine and 30% for bone. The results for young bone are in reasonable agreement with the radial immunodiffusion measurements (Table 3). The amount of EV albumin in kidney and intestine is similar in young and adult animals and in skin, muscle and bone it is higher in young animals.

*Egress of albumin.* The mass of albumin entering extravascular tissue/g tissue.unit time can be calculated from the initial slope of the curves for the tissue:plasma ratio plotted against time (right hand graphs, Figs. 4–8). These graphs were drawn using intravascular plasma volumes calculated from $^{51}$Cr-erythrocyte measurements. Plasma radioactivity at the time of death was used but the error due to this is not appreciable since fall-off is less than 10% in the first 30 min. In all tissues the exit of albumin is more rapid in the young than in the adult. Except for kidney, the points during the first forty minutes are fitted approximately by a straight line through the origin. In kidney exit of albumin from vessels is very rapid and the initial slope is estimated using the earliest point at 5 min. Similar graphs (not shown) have been drawn assuming that the intravascular volume in the tissue is equal to the 5 min fibrinogen volume using the results in Table 1*c*. The effect of this is to lower the earliest point proportionately more than the later time points. For young rabbits the results for the egress of albumin (mg/g.hr) have been calculated from the initial slopes from both sets of graphs (Table 3, 3*a* and *b*).

## DISCUSSION

*Extravascular albumin in different tissues.* Our values for soft tissues are similar to previous results obtained for rats (Katz *et al.* 1970). In recent work the total albumin in young bone was found to be about 2·8 mg/g (Triffitt & Owen, 1973). Hence the amount of extravascular albumin per gram of bone is comparable with the maximum values for soft tissues (Table 3) particularly when allowance is made for the greater density of bone (about 2·0 g/ml.). From the results in Table 3 it can be calculated that about 27% of the albumin in bone is in tissue fluid, 16% is intravascular and the rest, 57%, is in calcified matrix. Only the albumin in tissue fluid in bone is exchangeable with plasma whereas in soft tissues the majority of extravascular albumin is exchangeable. The rate of clearance of the albumin in extravascular tissue fluid in bone is approximately once every hour. This is more rapid than in skin and muscle, comparable with intestine and less rapid than in kidney (Table 3).

*Egress of albumin.* Measurements of rates of egress vary by a factor of two to three times depending on the method used to determine the intravascular plasma volume in the tissue (Table 3). However, the relative values obtained for the different tissues are consistent with the morphology of their capillary endothelium, being more rapid in kidney and intestine where large fenestrations have been observed and less rapid in skin and muscle where a continuous basement membrane is present (Karnovsky, 1968), the value for bone, which has not been measured before,

is similar to other tissues (Table 3). Our results are also the same order of magnitude as values hitherto obtained by completely different methods. For example, from measurements of the rate of lymph flow and the lymph: plasma concentration ratio the over-all rate of egress of albumin from capillaries in the dog paw was found to be 2·2 ml. plasma/100 g.hr (Garlick & Renkin, 1970) which is comparable with our results for skin, muscle and bone, which range from about 0·3–3·0 ml./100 g. hr in young rabbits.

The small amount of non-precipitable radioactivity at early times (Table 2) was neglected in calculations of egress rates of albumin. This is justified if it is due mainly to catabolism within the tissue (see next paragraph). However if equilibration of plasma free iodide with EV space is assumed this would account for about 5–10% of the EV radioactivity found in kidney, about 5% in bone, about 20% in intestine and muscle and about 70% in skin, consequently in this event the value for egress rate in skin may be seriously overestimated.

*Degradation of albumin.* Sites of albumin catabolism are not definitely known though recent evidence suggests that this occurs in the vascular endothelium of tissues (Parving, Rossing & Jensen, 1974). In the present experiments the proportion of non-precipitable radioactivity appears to increase with time after injection in kidney, intestine and bone suggesting some break-down of albumin in these tissues, though the extent to which this non-protein bound radioactivity can be correlated with albumin break-down *in vivo* needs to be investigated.

*Incorporation of albumin into calcified matrix.* In our laboratory a 400 g rabbit increases body mass due to growth by about 5% per day. Assuming that bone, without marrow, vessel contents and cartilage, is 8% body weight and that calcified matrix contains about 1·6 mg albumin/g bone, as calculated above, then about 2·6 mg albumin is added to calcified matrix per day in the process of bone growth. By using the results in Table 3, column 3, it can be shown that about 670 mg albumin enter extravascular tissue in bone per day. Hence, even in actively growing rabbits, less than 0·5% of the albumin which enters bone tissue fluid per day is incorporated into calcified matrix. Consequently the uptake of albumin into bone matrix does not preclude its use as a tracer for studying the turnover of albumin in the tissue fluid of bone.

*Extrapolation to total tissue.* The values obtained for small samples of tissue have been extrapolated to give approximate values for total tissue. In young rabbits of 400 g body weight, the kidney mass is 5 g and assuming bone is 8% of body weight and the percentages for other tissues given by Katz *et al.* (1970), the amount of extravascular albumin and egress of albumin for total tissue have been calculated (Table 4). About two thirds

of total body albumin is extravascular and the majority is in skin and muscle (column 2, Table 4) in agreement with the findings of many workers.

The half life of $^{125}$I-albumin in plasma is about 3 and 5 days in young and adult rabbits respectively (Fig. 1) in agreement with other work (Hoffenberg, 1970; Anker, 1960). In adult man the half-life is about 20 days and an amount of albumin equal to the intravascular plasma albumin mass enters extravascular tissue about once daily (Parving *et al.* 1974). From Table 4, column 3, it can be calculated that in the young rabbit an amount equal to 8 times or more the intravascular plasma albumin mass leaves the vessels per day and this is consistent with the higher turnover and metabolism of plasma protein in small animals (Munro, 1970).

TABLE 4. Results for total tissue extrapolated from measurements on small samples. Tissue weight (% total body), extravascular albumin in total tissue (mg), egress of albumin from intravascular to extravascular space in total tissue (mg/hr) in young rabbits 400 g weight

| | 1 | 2 | | 3 | |
|---|---|---|---|---|---|
| | % by weight of total body | EV albumin (mg) | | Egress of albumin (mg/hr) | |
| | | *a* | *b* | *a* | *b* |
| Kidney | 1·25 | 33 | 21 | 156 | 51 |
| Intestine | 5 | 86 | 79 | 60 | 34 |
| Skin | 16 | 435 | 421 | 70 | 26 |
| Muscle | 60 | 408 | 396 | 134 | 67 |
| Bone | 8 | 26* | 23* | 34 | 21 |
| Rest | 6·25 | | | | |

Plasma is 3·5 % of body weight and contains 520 mg albumin.
* mg albumin in bone tissue fluid.
*a* and *b* same as in Table 3.

In conclusion, in analogy with other tissues the extravascular tissue fluid of bone probably contains a spectrum of plasma proteins which circulate through it as has been found here in the case of albumin. It is likely that plasma proteins act as carriers for hormones, vitamins and other metabolites and play a role in the regulation of osmotic pressure in bone tissue. Plasma proteins are present in interstitial fluid adjacent to periosteal and endosteal surfaces, along the walls of haversian canals and throughout the canaliculae and lacunae (Owen *et al.* 1973; Cooper, Milgram & Robinson, 1966; Matthews, Martin, Kennedy & Collins, 1973). The surfaces of bone and the walls of the canaliculae present a large area of mineral which might be available for exchange and the passage of tissue fluid through this region must play a role in mineral homoeostasis. Plasma proteins may also be important for the control of the microenvironmental

pH in bone by acting in a buffering capacity (Neuman & Bareham, 1975). Whether any plasma proteins have specific roles in bone metabolism is not known although the recent finding (Ashton, B. A., Hohling, H. J. and Triffitt, J. T. submitted for publication) that a plasma $\alpha$-glycoprotein (identified as the $\alpha_2$ HS glycoprotein) is uniquely concentrated in calcified tissue during the process of bone formation raises interesting possibilities.

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## REFERENCES

ANKER, H. S. (1960). *The Biosynthesis of Plasma Proteins in the Plasma Proteins*, vol. II, ed. PUTMAN, F. W., p. 267. New York and London: Academic Press.

ASHTON, B. A., TRIFFITT, J. T. & HERRING, G. M. (1974). Isolation and partial characterization of a glycoprotein from bovine cortical bone. *Eur. J. Biochem.* **45**, 525–533.

COOPER, R. R., MILGRAM, J. W. & ROBINSON, R. A. (1966). Morphology of the osteon. *J. Bone Jt Surg.* **48**, 1239–1271.

GARLICK, D. G. & RENKIN, E. M. (1970). Transport of large molecules from plasma to interstitial fluid and lymph in dogs. *Am. J. Physiol.* **219**, 1595–1605.

HOFFENBERG, R. (1970). Control of albumin degradation *in vivo* and in the perfused liver. In *Plasma Protein Metabolism*, ed. ROTHSCHILD, M. A. & WALDMANN, T., p. 239. New York and London: Academic Press.

KARNOVSKY, M. J. (1968). The ultrastructural basis of transcapillary exchanges. *J. gen. Physiol.* **52**, 64S–95S.

KATZ, J., SELLERS, A. L., BONORRIS, G. & GOLDEN, S. (1970). In *Plasma Protein Metabolism*, ed. ROTHSCHILD, M. A. & WALDMANN, T., pp. 129–154. New York and London: Academic Press.

MCFARLANE, A. S. (1964). In *Mammalian Protein Metabolism*, vol. 1, ed. MUNRO, H. N. & ALLISON, J. B., pp. 297–341. New York and London: Academic Press.

MANCINI G., CARBONARA, A. O. & HEREMANS, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235–254.

MATTHEWS, J. L., MARTIN, J. H., KENNEDY, J. W. & COLLINS, E. J. (1973). An ultrastructural study of calcium and phosphate deposition and exchange in tissues. Hard tissue growth, repair and remineralization. *Ciba Fdn Symp.* **11**, 187–211.

MUNRO, H. N. (1970). Factors in regulation of liver protein synthesis. In *Plasma Protein Metabolism*, ed. ROTHSCHILD, M. A. & WALDMANN, T., p. 157. New York and London: Academic Press.

NEUMAN, W. F. (1969). The milieu interieur of bone: Claude Bernard revisited. *Fedn Proc.* **28**, 1846–1850.

NEUMAN, W. F. & BAREHAM, B. J. (1975). Evidence for the presence of secondary calcium phosphate in bone and its stabilization by acid production. *Calc. Tiss. Res.* **18**, 161–172.

OWEN, M., TRIFFITT, J. T. & MELICK, R. A. (1973). Albumin in bone. Hard tissue growth, repair and remineralization. *Ciba Fdn Symp* **11**, 263.

PARVING, H. H., ROSSING, N. & JENSEN, H. A. E. (1974). Increased metabolic turnover rate and transcapillary escape rate of albumin in essential hypertension. *Circulation Res.* **35**, 544–552.

REGOECZI, E. (1974). *Fibrinogen in Structure and Function of Plasma Proteins*, vol. 1, ed. ALLISON, A. C., p. 133. London and New York: Plenum Press.

RENKIN, E. M. (1964). Transport of large molecules across capillary walls. *Physiologist, Wash.* **7**, 13–28.

STUDER, R. & POTCHEN, J. (1971). The radioisotopic assessment of regional microvascular permeability to macromolecules. *Microvasc. Res.* **3**, 35–48.

SWAN, H. & NELSON, A. W. (1971). Blood volume measurement: concepts and technology. *J. cardiovasc. Surg.* **12**, 389–401.

TRIFFITT, J. T. & OWEN, M. (1973). Studies on bone matrix glycoproteins. *Biochem. J.* **136**, 125–134.